COMPARISON OF ESCHERICHIA COLI AND STREPTOCOCCUS FAECALIS AS A TEST ORGANISM TO DETERMINE THE SANITARY QUALITY OF FOOD

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THESIS

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COMPARISON OF ESCHERICHIA COLI AND STREPTOCOCCUS FAECALIS AS A TEST ORGANISM TO DETERMINE THE SANITARY QUALITY OF FOOD.

by

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Introduction

The need for a bacteriological test for the sanitary conditions of food has long been recognized. Early investigators searching for such a test recognized certain merits in the normal inhabitant of the intestines, Escherichia coli. This organism, while normally not pathogenic, was sufficiently hardy to survive a variety of physical and chemical conditions so as to make it of value as a test organism. As data on food borne infections and epidemics accumulated it became apparent that many of them were directly traceable to contamination of the food by pathogenic, enteric bacteria such as the paratyphoid, typhoid, paradysenteriae, and dysenteriae groups.

It was found that bacteria gained entrance to the food in a variety of ways such as through water, soil, food handling by people either sick with an intestinal disease, or healthy carriers, or by those careless in their personal habits, by flies, insects and rodents. Since many of the enteric pathogens were none too hardy after they left the body and some of them were difficult to grow on many media or could not compete with other organisms on artificial media, it became apparent that the more robust E. coli was more nearly the perfect test organism than any of the others found in the intestines.

Later, complications arose when it was discovered that

a closely associated organism, Aerobacter aerogenes, was also found in the intestines, in water and in food. This difficulty was overcome when methods were found to distinguish between the two different organisms. It was also found that the normal habitat of the Aerobacter group was in the soil and on foods rather than in the intestine.

One of the more recent criticisms of E. coli as an index of contamination is its hardiness, a factor which previously recommended it. Some advocate a less viable organism which is also an inhabitant of the intestine, Streptococcus faecalis, as a better index of contamination since its presence is indicative of present or more recent contamination.

The present study was to determine the viability of E. coli and Strept. faecalis on various foods having a different nutritive and chemical composition, a wide range of pH values, different consistencies, and moisture content. Such a study should help to determine the suitability of each to serve as a satisfactory test organism for food contamination.

Review of Literature

It is said that Aristotle noted that sickness and death lived in the air, and in the water the people drank, and that he considered cleanliness a factor in long life.

The ancient Romans recognized the importance of sanitation in that the position of Sanitarian was given to an important person, generally a member of the senate and that under his direction contamination of the water supply by an individual was punishable by death in the arena. It took centuries and a great deal of work to lay the scientific foundation for their ideas and theories.

Some excellent bacteriological work had been done in the years preceeding 1902 along samitation lines, but it wasn't until after this that Papastiriu (1902), Metcalf (1905), Prescott (1906),.Winslow and Walker (1907), Fromme (1910), Rogers, Clark and Evans (1914), Johnson (1916) and others published work on lactose fermenting organisms that interest began to grow. Rogers, Clark and Evans (1915) claimed through investigation that bacteria identical with the colon type frequently appeared on grains, fruits and grasses. The work of Rogers,and Clark (1917) further stimulated the interest in the coliform group. The correlation of specific characters of members of this group with their habitat had been sought for some time on account of its sanitary significance but until this time no progress had been made which attracted much attention.

Koser (1915) published the work done using simple nitrogen compounds for utilization by microorganisms as a differential method of identification. Levine, Weldin and

Johnson (1917) were also working on differentiating between coli-like organisms. Chen and Rettger (1920) identified B. aerogenes and B. coli in their soil relationships.

The great development in the field however, is closely associated with media and methods and the use for which they were developed. Water was the first of these fields and in 1904 it made its nucleonic bid through the American published book "Elements of Water Bacteriology, with Special Reference to Sanitary Water Analysis". At that time methods for the sanitary analysis and significance had only begun to crystallize, yet work in the field progressed rapidly under the stimulation of the Committee on Standard Methods of the American Public Health Association. Through revision and trial, new methods were devised and improved and interpretation became standardized.

Prescott, Winslow and McCrady (1946) felt that in water analysis the presence of moderate numbers of coliform organisms should not be considered a sure sign of dangerous pollution but rather should serve as an indication of possible pollution, and search for the source should be the prime factor. Coliform organisms when present, however, did present a reasonably accurate index of the amount of pollution.

Although the significance of the streptococci as sewage organisms was not established with the same definiteness

which marks our knowledge of the coliform group, these bacteria had been isolated so frequently from polluted sources and so rarely from normal waters that it seemed reasonable to regard their presence as indicative of pollution. Originally reported by Laws and Andrews (1894), their importance was not emphasized until Houston (1899-1900) laid special stress upon the fact that streptococci and staphylococci seemed to be characteristic of sewage and animal waste. Laws (1894) believed that they were more truly indicative of dangerous pollution, since they were readily demonstrated in waters recently polluted and seemed altogether absent in waters above suspicion. Horrocks (1901) found these organisms in great abundance in sewage and in waters which were known to be sewage polluted, but which contained no trace of E. coli. He found by experiment that E. coli gradually disappeared from specimens of sewage kept in the dark at the temperature of an outside veranda, whereas the commonest forms which persisted were varieties of streptococci and staphylococci. On the whole, there can be no doubt of the fact that streptococci occur on the surfaces of the human and animal body more commonly than anywhere else in nature. Mallman and Litsky (1950) believe that enterococci would appear to be good indicators of public health hazards from sewage in soils and on vegetables. They found that the streptococci dies out of soil samples much more quickly than did E. coli

and the organism Strept. faecalis persisted longer than S. typhosa.

Burton (1949) suggested that the enterococci might prove superior to the coliform organisms as an index of fecal contamination in frozen foods as fecal streptococci were most likely to survive the storage temperature, although the coliform seemed to be the best test before freezing and storing. Burton studied frozen vegetables and cantelopes.

Although Washburn (1910) reviewed the history of frozen and iced products, and Buchan (1910) made a complete bacteriological study of English ice cream plants finding large percentages of coliform organisms, the attention paid to their sanitary qualities seemed to have been embrological until the 1930's. Hammer (1912) showed that large numbers of bacteria, which he did not identify, existed in ice cream. Ayers and Johnson (1915) worked on a synthetic medium for the determination of colon bacilli in ice cream. They found gas forming bacteria of the coliform group in 88 percent of the cases examined. Although various media were used, it was concluded that there was no entirely satisfactory method of detection known at that time.

Fabian and Coulter (1930), Jenkins (1926), Brannon (1931), Newman and Reynolds (1930), Smallfield (1933) and Prucha (1936)(1939), noted the significant factors that (a) coliform organisms will appear in the manufactured

product if not eliminated in the dairy product, (b) that a pasteurizing temperature of 65.5°C. was better than 62.8°C. for 30 minutes, (c) that the protective action of fat and succrose were slight, (d) checks for E. coli should be instituted to check plant procedures, (e) that additives to ice cream mix should be watched as coliform entered through the addition of nuts, coloring matter, and fruits.

Fabian (1935) discussed the error of manufacturing the mix one place and freezing elsewhere. Fountain service at the retail level was also found to be a source of contamination as were glassware, dippers, syrups, spoons, and dipper water as studied by Fabian and Hook (1936-1937). Work done on factory packed ice cream (1942) showed that 25 percent of the samples tested were outside of the safety standards set up of ten organisms per ml. of sample. Many other types of frozen desserts had high coliform count, many being of the fecal type. The suggestion was advanced by Fabian (1937) that the FDA take over inspection of plants and material where interstate shipment is involved. There definitely existed a need for control based on the coliform index as seen by a review of early literature.

Where it is the aim of water technicians and sanitary engineers to eliminate every trace of contamination with fecal material of which E. coli is the indicator, the presence of this organism in milk must be viewed in a

different light. Although it is recognized that it is impossible to keep raw milk free of E. coli, the contamination of this product after pasteurization should lead to serious suspicions of improper technique or equipment somewhere in the plant.

A low coliform bacterial count in finished potable water lacking nutritive value would be more significant than high numbers of like bacteria in milk which is an excellent medium for their growth.

Smit, Krol and van Wijk (1939) stated that the time element in bacteriological analysis of milk was an important factor and worked on media to hasten diagnosis. The field has progressed far in the eleven years since 1939, and new methods and media brought into common usage since their article show the rapid and fluctuating progress in the field.

Seven important elements, as listed by the Diversey Corporation (1950), which may prove factorial in coliform recontamination which may apply to food products are: (a) Equipment-design, construction and repair, (b) physical conditions in the plant--exposure of equipment and processing operations to contamination by dust from the exterior, or drip of condensate, (c) exposure to insect contamination (d) operating practices which favor recontamination, (e) unhygienic practices of personnel, (f) incomplete cleaning of equipment, (g) ineffective bactericidal treatment of

equipment.

Although the work done in the field of milk sanitation and even more specifically in relationship to the coliform group has been great in volume, and although the different tests devised and utilized are numerous, a general summary has been made in the Diversey Corporation bulletin (1950). "(1) Limitation of the coliform content of pasteurized milk is becoming more general and widespread. (2) Coliforms are widely distributed in nature, and--at least currently, constitute a greater or small, but consistent portion of the bacterial content which is normal to raw milk. (3) It is the consensus of qualified opinion that coliforms are destroyed by effective commercial pasteurization, at least to the extent that survivors are so few as to escape detection. (4) The corollary of this view is that the presence of coliforms in freshly pasteurized milk constitutes a reliable index of post-pasteurization recontamination, potentially with bacteria more pathogenic than coliforms. (5) Sources of coliforms, and causes and avenues of contamination, and control measures, are of primary interest in improving milk sanitation."

Grimes (1934) made a study of the action of certain microorganisms in relation to the keeping quality of butter in storage and (1934) noted the importance of the presence of the coliform group and the purity of the water used in

the process. He advocated use of the standard lactose broth method of water analysis to ascertain this. Hammer and Yale (1932) found that in 10 days at about 18°C., both Escherichia and Aerobacter species grew in salted as well as unsalted butter.

Long, Hendrick and Hammer (1944) stated that heat resistant Escherichia cultures existed and dairy products should be tested for heat resistant organisms before assuming that pasteurization was inadequate or that contamination had occurred. The relationship of coliform organisms to pasteurization and higher temperatures was studied by many including Ayers, Henry and Johnson (1915), Stark and Curtis (1936), and Tanner and Windsor (1929).

Pecan meats were found by Ostrolenk and Welch (1940) to be heavily contaminated with coliform organisms which originated from picker's hands, picking table and picking pans. The use of metal instead of wood reduced the count, as did closer supervision of the personnel.

Faville, Hill and Parish (1950) found bacteria to be the predominating microorganisms present in concentrated orange juice, and that they died out quickly after storage. E. coli was significantly reduced in numbers at low temperature storage.

The development of methods of coliform detection has been based on the progress in the field of media. In 1922,

lactose broth, with confirmatory Endos or litmus medium, was brought into use with 1.0, 0.1 and .01 ml. shellfish samples to detect the presence of lactose fermenting organisms, and thereby detect sewage contaminated beds. This early search was a huge step in the setting up of a method and indices for searching out possible danger points for enteric disease producers. Varieties of experiment medium were numerous, but one of the great developments coming out of the period up to 1934 was the most probable number for evaluation of coli-aerogenes test by the fermentation tube method by Hoskins. Leifson (1935) devised culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. Stark and Curtis (1936) considered the advancement in the field of media to be sufficient to warrant a series of tests to evaluate certain media for the detection of colon organisms in milk, and Mallmann (1939) made a study of media for coliform organisms. Mallmann and Darby (1941) developed the formula for lauryl tryptose broth, a medium which was recommended for use in the standard tests for the coliform group as specified in "Standard Methods for the Examination of Water and Sewage" of 1946. Workers in the field of water bacteriology compared lactose broth and lauryl tryptose broth and found the latter superior in that it gave fewer false positive presumptive tests and suppressed the

spore forming aerogenic bacteria.

The Journal of Bacteriology (1946) surveyed a more rapid method for detecting coliform bacteria in natural waters and shellfish. This method makes use of a sodium lauryl sulfate tryptose nitrate broth and it reduces the time required for the presumptive test for coliform bacteria in water to twelve hours or less. Data obtained from sea water, crab meat, and oysters showed that nitrate positive tests were obtainable within eight to ten hours and that it was unnecessary to confirm these tests.

Mallmann and Seligmann (1950) stated that media for streptococci detection should be selective so that a clouding of the liquor would be indicative of the presence of streptococci. Lack of comparative studies did not permit an accurate determination of the best medium. Limited studies, however, indicated that azide dextrose broth was superior to lactose broth, Strept. faecalis broth (SF), or azide broth. Azide dextrose broth has the property of inhibiting the gram-negative bacteria. Mallmann and Litsky (1951) used various azide broths and SF broth in their tests of soils for the presence of enteric organisms. They believed azide dextrose broth superior to other azide media and noted, that as some spore forming gram-positive rods grow in this medium, Gram stains were necessary for accurate determination.

Foods contain not only nutritive elements for microorganisms, but inhibitory agents in the form of salt, sugars and organic acids. Many of these agents have long been recognized. Vinegar (acetic acid), sour milk and sauerkraut (lactic acid) have been used as natural preservatives since ancient times. The specific action of the acids, as summarized from the work of Reid (1932), Levine and Fellers (1940), Nunheimer and Fabian (1940), and McCulloch (1945) may be due to one or more of the following: (a) the effect of the hydrogen ion concentration, (b) the effect of the characteristic anion, (c) the effect of the undissociated molecule, and (d) the effect of surface tension.

The germicidal and preservative properties of the strongly disassociated acids were closely correlated with the hydrogen-ion concentration which they produced. The addition of sufficient amounts of these acids will so reduce the pH of the medium that cells could no longer multiply and a static condition was produced. With the addition of more acid, the toxicity of the hydrogen-ions became so great that the bacteria were killed. The germicidal efficiency of the rarely used, strong acids was markedly increased by increases in temperature.

Winslow and Lochridge (1906) concluded that the mineral acids were fatal in concentrations in which they were highly dissociated, their action running parallel, not to their

normal strength, but to the number of free hydrogen ions per unit of volume. The effect appeared to be due to the whole molecule and was specific for each acid. S. typhosa was found to be two to four times as sensitive to hydrochloric, sulfuric or benzoic acids as E. coli. Nunheimer and Fabian (1940) found a difference in the action of the various organic acids upon one and the same strain of staphylococci which they believed to be due to a specificity of reaction. Paus (1908) showed that the weak fatty acids and the dibasic organic acids were very strongly inhibitory against E. coli and E. typhosa. Reid (1932) found that the monobasic acids, the least dissociated of the acids used, inhibited growth at a much lower hydrogen-ion concentration than the strongly dissociated acids such as oxalic. In his scheme showing the bactericidal action of organic acids against various organisms exposed for 15 minutes at 20 degrees C., the degree of toxicity ran in order of acetic acid first then lactic and citric acid. Levine and Fellers (1940) found that acetic acid was inhibitory at a pH of 4.9 to 5.0 with .04 to .03 percent inhibiting acidity, and with lethal pH's of from 4.5 to 4.9 and .04 to .09 percent lethal acidity for bacteria. Acetic acid was found by Nunheimer and Fabian (1940) to be the most actively inhibitory and germicidal member against food poisoning staphylococcus. They listed the decreasing order of the bactericidal action of the acids as acetic,

citric, lactic, malic, tartaric and hydrochloric, while the decreasing order of the inhibiting or bacteriostatic action was found to be acetic, lactic, citric, malic, tartaric, and hydrochloric.

Cultures Used

Escherichia coli strain communior, communis and O-111 were obtained from the Michigan State Health Laboratory, Lansing. Strain O-111 is of noteworthy importance in that it is credited with causing the often fatal infant diarrhoea. Culture ATCC 9637 was obtained from the American Type Culture Collection, Washington, D.C. A strain of E. coli which had been isolated from the rumen of the cow was obtained from our own department. Another strain of E. coli was isolated and purified from human feces and water samples. The culture from the human source was assigned the number HS-04. The culture obtained from water was assigned the number W-52950.

All strains were rechecked before using by the dilution plate method using eosin-methylene blue agar and transfering single isolated typical colonies to lauryl tryptose broth. All transferred colonies were gas positive in lauryl tryptose broth and staining showed all organisms to be gram-negative short rods.

Viability of the E. coli was built up by seven daily consecutive transfers into a peptone broth base which

consisted of:

beef extract	3 gms.
peptone	5 gms.
lactose	5 gms.
water	1 liter.

The last broth culture of the daily transfers served as the inoculum to test the viability of the different strains in the foods studied.

Strept. faecalis cultures 1325 and 6057 were obtained from the American Type Culture Collection, Washington, D.C. These cultures were inoculated into semi-solid preparations of tryptose blood agar base which was made up as follows:

beef extract	3 gms.
tryptose	10 gms.
sodium chloride	5 gms.
agar	7 gms.
water	1 liter.

Before inoculating into food, the organisms were transfered daily for five days into broth made according to the above formula by eliminating the agar.

Experimental Work

The first set of canned foods studied were those commonly used in the household which had a wide pH range. The cans were opened aseptically and foods of large particle size were ground in a sterile Warring blender. Approximately 75 ml. portions were placed into dilution bottles and autoclaved for 15 minutes at 121°C.

The pH was determined with the Cenco pH meter using

a glass and calomel electrode combination. A 1.0 ml. sample plate check was made to determine the presence of mesophilic and thermophilic bacteria using tryptone glucose extract agar and nutrient agar. The autoclaved food was then inoculated with an actively growing 24 hour culture of E. coli strain communior. The food was then plated in serial dilutions to obtain the initial inoculum of bacteria per ml. Distilled water blanks and tryptone glucose extract agar were utilized for this procedure.

The semi-solid food and the first 99 ml. dilution blank to which one ml. of sample was introduced were shaken with a mechanical shaker oscillating 180 times per minute. The best mixing was obtained by having the bottles in a horizontal position with the long axis of the bottle in line with the direction of shaking. All subsequent decimal dilutions were shaken manually.

Five agar plates were prepared for each dilution. Three plates out of the set of five were prepared by placing a thin layer of TGE agar upon the bottom of the sterile petri plate and allowing it to harden before the dilutions were added to the plates. The dilution water containing the food was then added and another thin layer of agar was poured into the plates. The two liquid elements were thoroughly mixed. The other two plates were made in the usual manner without a base layer of agar.

They were shaken like the first set of three plates. It was found that there was closer corelation between the counts in those plates to which a thin base layer of agar had been added first, since they gave more uniform counts than plates made in the usual manner.

Colonies were counted on the Quebec colony counter after a three day incubation period at 30°C. The graphic results are given in Figures 1, 2, 3, and 4.

The second set of foods was run in a slightly different manner. Foods were placed aseptically in sterile dilution and wide mouth type bottles. These foods were not autoclaved, but controls were run with each food using lauryl sulfate tryptose broth and lactose broth. Each type of food was then inoculated with seven strains of E. coli using 0.2 ml. of the 24 hour culture prepared as described previously. They were incubated at 30°C. and each day for seven consecutive days a 0.1 to 1.0 ml. sample of liquid food and 0.2 to 1 gm. sample of dry solid food were inoculated into lauryl sulfate tryptose broth and lactose broth in which inserts were placed. The minimum transfer was used at the beginning of the seven day test and when it was seen that the percentage of gas at 12 hours was decreasing, a larger inoculum was used. More inoculum was used for the dryer foods and a lesser amount for foods with liquor present. The liquor from semi-solid

foods seeded with E. coli were transfered to the broth medium by pipetting. Examples of this type of food are peaches with syrup, tomato with juice, apricots with syrup, apple-sauce and hominy. Solid foods such as beans, meat and sauerkraut were weighed to determine the relative amounts in 1 and 0.1 gram samples respectively and approximate amounts used for inoculum.

Prior to transfer of the inoculated foods into the two broths all samples were shaken for 10 minutes at 180 oscillations per minute. After 16 and 36 hours incubation, the percentage of gas present in the insert vials of the tubes were read and charted in Tables 3 to 15.

Positive tubes were confirmed by using the confirmatory test which is used for water samples. This test was initiated on the first, third and seventh days. All brilliant green bile broth tubes, which were inoculated with three standard (4mm) loops of lauryl sulfate tryptose broth, yielded confirmatory tests.

A duplicate set of the same twelve foods which were inoculated with Strept. faecalis and transfers of the foods were made daily for seven consecutive days into dextrose azide broth. Turbidity was read at 16 and 36 hours at the beginning, but since the 36 hour reading gave the best results, the 16 hour reading was discontinued. A gram stain of the broth was made at three days and studied.

Results

The growth curve of E. coli var. communior was influenced by the medium in which it grew. This is illustrated in the graphs shown in Figures 1, 2, 3, and 4.

Hominy with a pH of 7.2 supported rapid growth of E. coli reaching a count of about one billion at 24 hours which was the greatest number in any of the foods tested. Figure 1 shows that this organism remained viable for a long time since there were four hundred thousand organisms per ml. still present at the end of 19 days.

Figure 1 also shows how E. coli grew in canned peas at a pH of 6.0. With an initial seeding of one hundred thousand organisms, they had increased to 96,000,000 in twelve hours. They reached their maximum numbers in 48 hours when the count was 240,000,000 per ml. They died off rapidly, reaching 42,000 in six days when mold contamination caused discontinuance of the experiment.

Corn, with a pH of 6.2 (Fig. 2), fostered quick growth of E. coli which increased from an initial number of 100,000 at two hours to 400,000 at 24 hours. The logarithmic decrease was gradual for the 15 days of the test at which time a count of 400,000 organisms per ml. still persisted.

Chicken soup with a pH of 6.4, (Fig. 2), showed bacterial increases of E. coli from the initial amount of

660,000 to 51,000,000 in six hours. At 15 hours the count had increased to 290,000,000. The number of bacteria remaining showed but slight variation from the first until 14th days when there was a gradual decrease. At the 7th day, 10,000,000 E. coli were present, but by the 14th day, the count had decreased to 140 organisms per ml.

E. coli was seeded into two samples of beef gravy (Fig. 3). One sample was inoculated with 75,000 organisms and the other with 750,000. The bacteria grew rapidly in both samples for the first 12 hours. Their numbers leveled off after reaching a peak of 40,000,000 and 77,000,000 respectively at 36 hours. 1,250,000 organisms remained viable in food determination one after 32 days, and 18,000,000 remained viable in determination two at 20 days. Organisms were present in large numbers up to 46 and 28 days when plating was discontinued. These data would indicate that the amount of the original inoculum influences the number of organisms which are subsequently present.

E. coli inoculated into tomato soup having a pH of 4.6, (Fig. 4), did not multiply to any great extent. The initial amount of one million for set one, and about 2,000,000 per ml. for the second set did not rise above 3,000,000 organisms per ml. upon incubation and were in a state of sharp logarithmic decrease at 12 hours. There was a general leveling off of this decrease after five days and less than

100 and 300 coliform organisms per ml. were present after 30 and 35 days when determinations in this food were terminated.

Inoculum in excess of 100,000 organisms per ml. reached the limit of their increases in from 24 to 48 hours. Of the foods used within the pH range of 4.6 to 7.2, all contained more than 10,000 organisms per ml. at the end of seven days incubation at 30°C.

Foods seeded with coliform organisms were inoculated into lauryl tryptose broth and lactose broth to determine the most promising common broth medium for rapid detection of coliform organisms. Lauryl tryptose broth gave 706 positive tubes to 630 for lactose broth. These results are the sum of 12 and 36 hour gas positive tubes. The foods in which E. coli produced the most gas at 12 hours and remained viable for the longest period of time in one ml. quantities were beef (Table 3), hominy (Table 4), beans (Table 5), peaches (Table 6), applesauce (Table 7), and tomato with juice (Table 8). The order was determined by calculating both lauryl tryptose broth and lactose broth fermentation with the production of gas at 12 and 36 hours.

Apricots (Table 9) showed a slowing of fermentation at 12 hours of incubation the third day after the food had been inoculated. Although the amount of food placed into the broth tubes was increased after the third day, the strain

of E. coli, W-52950, isolated from a contaminated water sample, and strain O-111 died out on the third and fifth day respectively. The fourth day after seeding the food, the coliform organisms were less viable since in the first 12 hours little gas was formed in the broths by any of the strains.

In orange juice (Table 10), with a pH of 3.5, the gas formation in the fermentation tubes lessened in quantity after it had been incubated one day. Gas appeared in lauryl tryptose broth from one to three days after the organisms failed to ferment the lactose broth. E. coli var. communis evidently was the hardiest strain of all since it was the only strain surviving to show continued fermentation after five days.

In potato salad (Table 11) with a pH of 4.8, 24 hours after food inoculation all strains of E. coli fermented both lauryl tryptose broth and lactose broth at the 12 hour interval. By the second day after food inoculation, it was necessary to incubate the broth tubes 36 hours to get gas formation and the third day, after mixing coliform bacteria with the food, only five of the seven strains gave positive results; on the fourth day only three of the seven. On the sixth and seventh days, a heavier inoculum of potato salad into the broth tubes yielded positive results in lauryl tryptose broth for the human strain HS-04.

In sauerkraut with a pH of 3.8, (Table 12), two strains of E. coli gave negative tests at one day, and five of the seven strains gave negative tests at two days. Only E. coli var. communis remained viable to the fourth day with a positive test in lauryl tryptose broth after 36 hours of incubation.

Mayonnaise with a pH of 3.7, (Table 13), proved to be very bactericidal, yielding six out of seven positive coli tests at one day, and only one positive coli test at two days after inoculation in the food. There were no positive tests after 48 hours even though the amount of food inoculated into the broth was increased.

Cranberry sauce with a pH of 2.8, (Table 14), showed positive tests after the first day and then in only five of the seven strains. There were no positive tests the second day with one gram samples, and although two grams of the material was used as inoculum into the broths the third day, all sampling remained negative.

The foods used fell into three general groups in regards to the viability of E. coli in them and Tables 3 to 15 are arranged accordingly.

Group I presents the best possibility for using tests for E. coli to determine the sanitary quality of food. In this pH range of 6.7 to 4.6, bacterial growth is favored while the inhibitive action of the organic acids is the least.

Organisms in this group of foods remained viable for the seven days of the test. Gas was discernible within 16 hours in the test broths. In group II foods with pH ranges of 3.5 to 4.8, E. coli organisms did not grow as well as they did in Group I foods. Organisms in Group III foods with pH's of 3.8 to 2.8 showed little gas production at 16 hours and yielded gas positive reactions for only a day or at the most, two days.

Strept. faecalis, as seen in Tables 15 and 16, remained viable for a longer period of time in mayonnaise and orange juice than did E. coli. Turbidity should not be used as a criterion for the presence or absence of streptococci. Non-turbid appearing tubes showed the presence of streptococcus when observed by Gram's stain. Applesauce and apricots appeared turbid, but Gram's stain revealed the presence of a large Gram positive bacillus. Aside from mayonnaise and orange juice neither the E. coli nor streptococcus method showed any advantage over the other in determining sensitivity of test, or longevity of organisms in the foods used.

Discussion of Results

Plate counts of E. coli inoculated into foods having a wide range of pH values showed that this organism remained viable in them for long periods of time. During this time

the food would show chemical changes or odor. It was found that in the foods with a pH 6.2 to 3.8, the total plate count of organisms did not exceed one billion bacteria per ml. The growth curve of the organism used varied from food to food. Obviously no one curve could be said to be the representative growth curve for E. coli when considering their relationship to the entire field of food products.

Positive tests for the presence of E. coli in food were obtained with reasonable rapidity using methods of coliform detection developed for water analysis. The lauryl tryptose broth and lactose broth utilization by E. coli proved to be more practicable in regards to the ease of observation, the use of a minimum of equipment, and quicker results than the present dextrose azide method and gram's staining to detect streptococci organisms. Lauryl tryptose broth proved to be superior to lactose broth in the more acid foods. Lauryl tryptose broth fostered a greater number of positive gas tubes when the coliform organisms were attenuated and showed a higher percentage of gas positive insert tubes at 12 hours than lactose broth.

The E. coli detection was limited to the presumptive and confirmed test used in finished water analysis.

Strept. faecalis showed no advantage in sensitivity and was more costly since it necessitated the use of a microscope, glass slides, staining materials, and time to

make and examine the stains after incubation. At times, better turbidity and subsequently better slides of gram-positive streptococci were obtained by using dextrose azide broth after the incubation time had been extended to three days. Compared with this, the coliform presumptive test took 12 to 36 hours, and the brilliant green bile broth confirmatory test an additional 12 to 24 hours. Minimal time is important in detection of contamination of consumable and perishable products.

It is felt that since a great proportion of the work would be done in the field, or with a minimum of laboratory equipment, the coliform test using lauryl tryptose broth would be the more practical test.

The use of foods having different pH's and nutritive values showed that either the coliform or streptococcus test may be used successfully to indicate unsanitary conditions.

An attempt was made to calculate a factor on fermentation with gas production to see if pH or total titratable acidity could be used to indicate the longevity of coliform bacteria in foods.

If a bubble of gas or more was present in the insert tubes at 12 hours, a value of two points was given to that tube. If gas was present only at 36 hours, one point was assigned to that particular tube. If no gas was produced,

the score was zero. The value thus determined for each tube was individually totaled at the end of seven days. Each total was added for each of the seven strains. The reaction total is the grand total of the seven strains for seven days in one broth. This yielded an individual number for lauryl tryptose broth and one for lactose broth for one food.

The reaction total of lauryl tryptose broth, using all seven strains of E. coli for the seven days was calculated for each food. The reaction total was also determined for lactose broth for each food. The reaction total of lauryl tryptose broth and lactose broth was added to get the total number depicting bacterial action for each food. This is called the "food factor".

By separating the foods where the food factor takes the greatest proportionate jump, we find we have a naturally occurring set of three groups of foods.

Table I

Food factors of various foods
grouped according to the magnitude of the factor.

Group I -

Food	pH	Total titratable acidity	Lauryl tryptose factor
Beef	5.9	.074N	93
Peach	4.2	.0498	93
Hominy	6.7	.0125	92
Bean, navy	6.2	.0664	92
Applesauce	3.6	.0581	88
Tomato	4.6	.0664	88

Food	pH	Total titratable acidity	Lactose factor
Beef	5.9	.074N	97
Hominy	6.7	.0125	94
Beans	6.2	.0664	89
Peach	4.2	.0498	86
Applesauce	3.6	.0581	83
Tomato	4.6	.0664	78

Food	pH	Total titratable acidity	Total lauryl tryptose-lactose factor.
Beef	5.9	.0747N	190
Hominy	6.7	.0125	186
Beans	6.2	.0664	181
Peaches	4.2	.0498	179
Applesauce	3.6	.0581	171
Tomato	4.6	.0664	166

Group II -

Food	pH	Total titratable acidity	Lauryl tryptose factor
Apricots	3.8	.0872N	52
Orange juice	3.5	.166	40
Potato salad	4.8	.0498	35

Food	pH	Total titratable acidity	Lactose broth factor
Apricots	3.8	.0872	41
Orange juice	3.5	.166	30
Potato salad	4.8	.0498	19

Food	pH	Total titratable acidity	Total lauryl tryptose lactose factor.

Group III -

Food	pH	Total titratable acidity	Lauryl tryptose factor
Sauerkraut	3.8	.0913	12
Mayonnaise	3.7	.1577	6
Cranberry sauce	2.8	.166	4

Food	pH	Total titratable acidity	Lactose broth factor
Sauerkraut	3.9	.0913	4
Mayonnaise	3.7	.1577	2.5
Cranberry sauce	2.8	.166	2

Food	pH	Total titratable acidity	Total lauryl tryptose lactose factor.
Sauerkraut	3.9	.0913	16
Mayonnaise	3.7	.1577	9
Cranberry sauce	2.8	.166	6

Since there is no correlation between pH and titratable acidity, it is apparent that E. coli is inadequate to serve as a basis to determine the longevity of coliform bacteria in food. However, they do serve to make a general grouping of foods to determine the longevity of E. coli.

Group I, because of high pH and correspondingly low organic acid content,readily permitted E. coli detection since these bacteria grow readily in these foods. The transitional group of foods is not as an ideal medium of growth as Group I, consequently small initial contamination in foods listed in Group II might not be detected. Finally Group III seemed to be still less suitable for the growth of the coliform organisms. In fact these foods were bactericidal to these strains of coliform bacteria. Gas positive tubes in Group III foods would indicate recent or an extra large E. coli contamination.

The data obtained with E. coli in mayonnaise were in agreement with that obtained by Wethington and Fabian (1950) who found that enterotoxigenic strains of food-poisoning Staphylococci remained viable in commercial mayonnaise having a pH of 3.8 for 96 hours. Species of Salmonella survived one hour or less in samples of mayonnaise. It is therefore evident that these foods are not the ideal habitat of intestinal organisms of this type.

A totaling of the number of gram-positive streptococcus tubes for the seven day test yields a different sequence for the foods from that of the E. coli scheme.

Table II

Showing streptococcus factor for various foods.

Food	pH	Titratable acidity	Streptococcus factor
Hominy	6.7	.0125	14
Beans	6.2	.0664	14
Beef	5.9	.074	14
Peach	4.2	.0498	14
Mayonnaise	3.7	.1577	14
Orange juice	3.5	.166	13
Tomato	4.6	.0664	12
Apricot	3.8	.0872	9
Applesauce	3.6	.0581	8
Potato salad	4.8	.0498	7
Sauerkraut	3.9	.0913	7
Cranberry sauce	2.8	.166	3

There is no correlation between the viability, and food factors of Strept. faecalis with pH or total titratable acidity.

Additional experimental work is needed to secure a more solid basis for defining degree of sanitary significance of positive reactions within a general grouping. Field samples should be taken and tests run for both Strept. faecalis and E. coli in order to establish a standard method and to better interpret the results. Each type of food to be indexed should be individually studied in the laboratory; carbon dioxide, oxygen and surface tension studies should be in relationship to the biochemistry of the organisms; and the shelf life of food types should be ascertained so as to permit limitations of work.

Summary

Studies of the growth curve of E. coli in a variety of foods indicated that the curve was dependent upon the food in which the organisms were grown and that the height of the curve was determined by the amount of initial inoculum.

More positive coliform tests were obtained using lauryl tryptose broth than with lactose broth. Lauryl tryptose broth gave more positive tests at 16 hours than did lactose broth.

E. coli var. communis showed slightly more viability in the foods than other strains of this organism. Strain O-111, credited with causing infant diarrhea, was the least viable.

Strept. faecalis remained viable longer in orange juice with a pH of 3.5 and mayonnaise with a pH of 3.7 than did any of the strains of E. coli.

Since time is essential in any bacteriological test for the sanitary quality of food, the presumptive and confirmatory tests of coliform organisms indicate the advisability of using the coliform test over the streptococci test. The coliform test also is to be recommended since a microscope, slides, and gram staining are unnecessary for the test as used.

Conclusion

From the standpoint of time and amount of equipment required, the Escherichia coli test is preferable to the Streptococcus faecalis test in determining the sanitary quality of foods.

Figure 1. Graph of growth curve of E. coli communior in canned peas pH 6.0. and hominy pH 7.2.

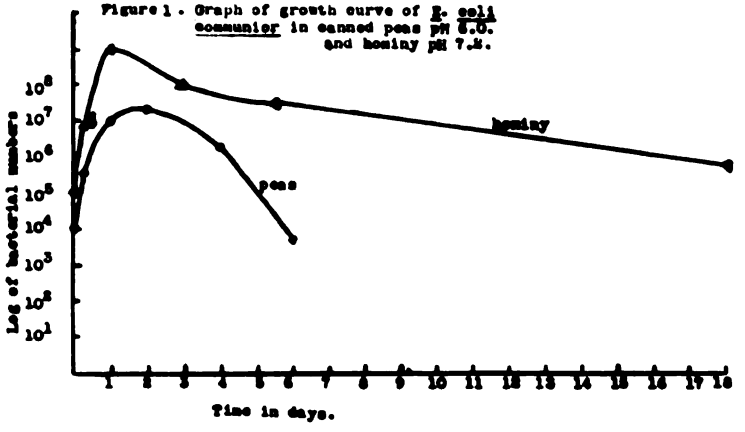

Figure 3. Growth curve of duplicate determinations of E. coli strain communior in canned beef gravy.

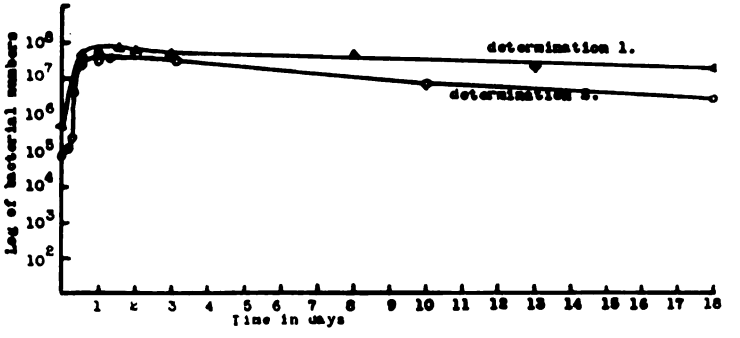

Figure 2. Graph of growth curve of E. coli communior in cream style corn pH 6.8. and chicken soup pH 6.4

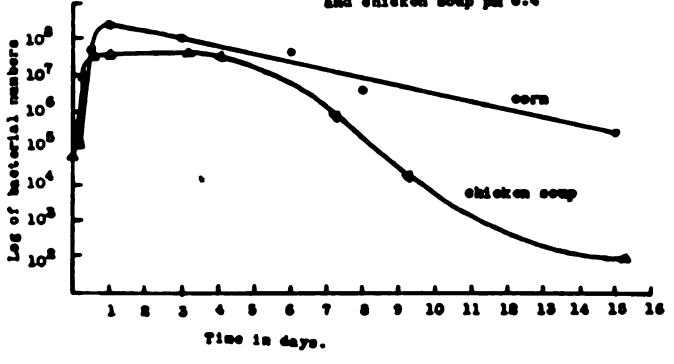

Figure 4. Growth curve of duplicate determinations of E. coli strain communior in canned tomato soup with a pH of 4.6.

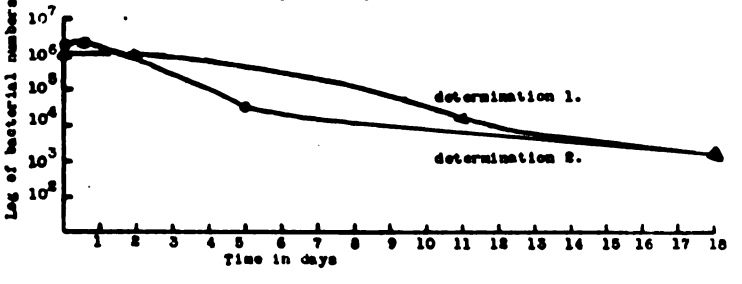

Table 3. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours for seven consecutive days. Broth was inoculated with canned beef seeded with *E. coli* with a titratable acidity of 0.0747 and a pH of 5.9.

Organism		Number of days after inoculation of food						
E. coli, strain:		1	2	3	4	5	6	7
-*communior*	12 LTB	50	4	2	35	20	1	70
	LB	10	2	5	1	15	7	10
	36 LTB	60	85	20	50	65	20	50
	LB	25	30	25	15	30	30	20
-*communis*	12 LTB	10	1	2	10	20	35	30
	LB	4	1	3	7	20	25	10
	36 LTB	60	75	40	40	70	75	60
	LB	15	35	25	25	30	50	20
*9637	12 LTB	25	sb	1	1	1	30	sb
	LB	5	1	3	1	1	15	20
	36 LTB	60	75	15	30	20	60	25
	LB	10	25	20	20	15	25	25
O-111	12 LTB	5	5	-	1	-	-	5
	LB	1	sb	5	5	1	2	1
	36 LTB	40	75	25	20	45	45	70
	LB	20	30	10	25	15	25	20
HS-04	12 LTB	50	1	2	1	5	2	2
	LB	7	2	3	5	5	1	7
	36 LTB	70	60	30	30	55	35	40
	LB	25	40	30	25	25	30	30
rumen cow	12 LTB	15	10	7	15	2	5	20
	LB	2	3	5	5	4	2	3
	36 LTB	60	75	55	60	40	75	50
	LB	15	40	25	25	40	30	25
W-52950	12 LTB	5	25	5	2	1	7	15
	LB	1	1	5	2	7	5	15
	36 LTB	75	80	50	55	45	75	70
	LB	10	25	30	25	40	35	40
Inoculum of food added to 10 ml. broth. (approximately)		.2	.2	.2	.5	1.	1.	1. gm.

*9637 - American Type Culture Collection strain
sb - small bubble.

Table 4. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours for seven consecutive days. Broth was inoculated with hominy seeded with E. coli with a titratable acidity of 0.0125N and a pH of 6.7.

Organism E. coli, strain		Number of days after inoculation of food 1	2	3	4	5	6	7
-communior	12 LTB	60	7	4	1	2	15	20
	LB	10	3	3	2	1	7	20
	36 LTB	95	60	30	50	60	50	40
	LB	15	40	5	20	25	20	30
-communis	12 LTB	40	3	1	50	1	5	3
	LB	7	3	5	5	1	3	7
	36 LTB	55	60	21	60	55	20	75
	LB	20	40	7	20	25	25	30
-*9637	12 LTB	12	2	1	2	1	45	15
	LB	7	2	3	1	1	20	sb
	36 LTB	20	45	30	3	20	50	30
	LB	15	15	20	2	15	20	20
O-111	12 LTB	15	sb	sb	1	sb	sb	1
	LB	10	sb	sb	2	sb	2	10
	36 LTB	75	30	20	17	60	20	50
	LB	25	10	15	15	10	15	25
HS-04	12 LTB	15	7	2	2	5	1	sb
	LB	5	2	4	2	2	1	2
	36 LTB	25	90	10	40	30	27	35
	LB	25	30	30	25	30	25	25
rumen cow	12 LTB	45	5	15	7	2	1	10
	LB	4	2	5	1	5	3	10
	36 LTB	50	50	20	30	20	35	50
	LB	15	25	10	10	20	20	20
W-52950	12 LTB	14	25	sb	2	2	15	20
	LB	7	2	1	3	7	15	15
	36 LTB	50	40	15	50	20	65	30
	LB	7	15	10	25	25	25	30
Inoculum of food added to 10 ml. broth.		.2	.2	.2	.5	1.	1.	1. ml.

Table 5. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours for seven consecutive days. Broth was inoculated with beans seeded with *E. coli* with a titratable acidity of 0.0664 and a pH of 6.2.

Organism *E. coli*, strain		Number of days after inoculation of food 1	2	3	4	5	6	7
-*communior*	12 LTB	50	5	25	1	1	5	30
	LB	1	3	1	-	1	1	sb
	36 LTB	55	85	60	20	25	45	70
	LB	10	35	15	7	25	25	10
-*communis*	12 LTB	50	1	sb	7	1	7	7
	LB	20	2	2	1	1	7	7
	36 LTB	65	65	30	5	25	55	25
	LB	25	40	15	25	20	40	25
9637	12 LTB	60	2	1	1	7	sb	sb
	LB	5	2	5	-	1	1	sb
	36 LTB	75	60	7	7	30	30	15
	LB	7	25	20	2	3	15	10
O-111	12 LTB	5	5	-	1	-		2
	LB	1	sb	1	sb	-		5
	36 LTB	65	75	2	35	7		70
	LB	45	30	5	25	20		35
HS-04	12 LTB	55	1	25	1	1	3	sb
	LB	5	1	2	sb	sb	-	1
	36 LTB	70	75	55	15	20	30	10
	LB	25	30	10	15	10	7	15
rumen cow	12 LTB	50	5	30	1	1	2	5
	LB	7	sb	1	1	sb	2	5
	36 LTB	65	80	75	25	40	70	30
	LB	20	35	7	10	15	30	25
W-52950	12 LTB	45	25	3	1	1	sb	20
	LB	2	1	2	sb	1	-	sb
	36 LTB	80	80	30	15	40	45	40
	LB	10	20	25	7	15	35	15
Inoculum of food added to 10 ml. broth. (approximately)		.1	.1	.1	.2	.5	.5	.5 gms.

Table 6. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours for seven consecutive days. Broth was inoculated with peach liquor seeded with E. coli with a titratable acidity of 0.0498 and a pH of 4.2.

Organism E. coli, strain		Number of days after inoculation of food 1	2	3	4	5	6	7
-communior	12 LTB	40	5	10	10	1	7	10
	LB	10	2	5	1	-	-	sb
	36 LTB	60	75	55	10	30	60	65
	LB	10	30	20	20	20	20	20
-communis	12 LTB	45	2	6	1	2	5	30
	LB	7	2	5	1	1	5	5
	36 LTB	75	75	50	20	45	50	60
	LB	20	30	30	20	30	45	35
9637	12 LTB	55	2	20	3	2	25	10
	LB	5	sb	10	5	2	sb	5
	36 LTB	80	75	55	55	30	50	50
	LB	25	25	30	20	20	20	25
O-111	12 LTB	20	sb	1	1	-	-	-
	LB	5	sb	1	sb	-	-	-
	36 LTB	75	30	40	40	40	-	25
	LB	40	30	25	20	1	-	20
HS-04	12 LTB	60	sb	1	1	1	-	1
	LB	10	1	2	1	1	50	40
	36 LTB	75	60	40	40	35	50	40
	LB	15	40	30	20	25	30	30
rumen cow	12 LTB	50	7	50	20	10	3	25
	LB	7	2	10	5	1	1	7
	36 LTB	75	70	80	45	50	40	75
	LB	15	40	25	20	25	35	25
W-52950	12 LTB	55	45	15	25	25	5	30
	LB	5	10	10	2	10	sb	-
	36 LTB	75	75	50	55	50	60	75
	LB	15	45	40	25	30	30	30
Inoculum of food added to 10 mls. broth.		.1	.1	.1	.15	.5	1.	1. ml.

Table 7. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours for seven consecutive days. Broth was inoculated with applesauce seeded with *E. coli* with a titratable acidity of 0.0581 and a pH of 3.6.

Organism *E. coli*, strain		Number of days after inoculation of food 1	2	3	4	5	6	7
-*communior*	12 LTB	60	2	7	25	sb	2	25
	LB	5	1	2	1	-	-	35
	36 LTB	80	99	60	75	75	75	75
	LB	10	40	25	20	25	30	40
-*communis*	12 LTB	10	2	6	2	1	10	30
	LB	2	4	5	1	1	2	15
	36 LTB	50	85	60	60	60	70	60
	LB	25	30	25	30	30	30	40
9637	12 LTB	60	2	10	1	-	sb	1
	LB	7	1	2	70	75	80	75
	36 LTB	80	90	70	70	75	80	75
	LB	20	30	30	25	20	25	25
O-111	12 LTB	15	7	sb	sb	sb	-	-
	LB	-	-	sb	-	-	-	-
	36 LTB	90	80	60	40	70	75	80
	LB	30	25	20	sb	2	-	5
HS-04	12 LTB	70	5	25	1	1	sb	7
	LB	12	1	7	2	1	-	1
	36 LTB	80	95	60	70	60	75	65
	LB	20	50	20	15	15	25	30
rumen	12 LTB	50	-	50	5	1	5	30
	LB	3	-	1	1	1	1	2
	36 LTB	80	80	80	50	70	70	75
	LB	15	30	30	20	30	25	25
W-52950	12 LTB	50	1	10	3	10	1	15
	LB	7	-	2	1	5	-.	sb
	36 LTB	75	90	70	50	60	70	75
	LB	10	30	25	25	30	70	35
Inoculum of food added to 10 ml. broth. (approximately)		.2	.2	.2	.2	.5	.5	.5 gm.

Table 13. Percentage of gas in lauryl tryptose broth and lactose broth inserts at 12 and 36 hours until negative results were secured for all *E. coli* strains. Broth was inoculated with commercial mayonnaise seeded with *E. coli*, and having a titratable acidity of 0.1577 and a pH of 3.7.

Organism		Number of days after inoculation of food					
E. coli, strain		1	2	0	1	2	3
-*communior*	12 LTB	-	-	1	-	-	-
	LB	-		2	-	-	-
	36 LTB	50	-	25	30	-	-
	LB	7	-	20	-	-	-
-*communis*	12 LTB	-	-	2	-	-	-
	LB	-	-	2	-	-	-
	36 LTB	-	-	40	25	-	-
	LB	-	-	25	-	-	-
9637	12 LTB	1	-	2	-	-	-
	LB	-	-	2	-	-	-
	36 LTB	60	-	20	30	25	-
	LB	25	-	20	7	-	-
O-111	12 LTB	-	-	1	-	-	-
	LB	-	-	-	-	-	-
	36 LTB	-	-	50	-	-	-
	LB	-	-	25	-	-	-
HS-04	12 LTB	2	-	1	-	-	-
	LB	-	-	1	-	-	-
	36 LTB	50	-	45	50	-	-
	LB	7	-	20	sb	-	-
rumen cow	12 LTB	3	-	5	-	-	-
	LB	-	-	1	-	-	-
	36 LTB	50	-	50	10	-	-
	LB	2	-	25	-	-	-
W-52950	12 LTB	-	-	2	-	-	-
	LB	-	-	1	-	-	-
	36 LTB	-	-	40	-	-	-
	LB	-	-	15	-	-	-
Inoculum of food added to 10 mls. of broth. (approximately)		.2	.2	.2	.5	1.	1. gm.

Table 15. Longevity of two strains of Strept. faecalis in foods of various pH's as determined presumably by turbidity and confirmed by Gram's stain.

Food	pH		ATCC 1325 Days 0	1	2	3	4	5	6	7	ATCC 6057 Days 0	1	2	3	4	5	6	7
Hominy	6.7	Turbidity at 36 hrs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Beans	6.2	Turbidity at 36 hrs	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
		Gram smear showing strept	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Beef	5.9	Turbidity at 36 hrs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato salad	4.8	Turbidity at 36 hrs	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
		Gram smear of strept.	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
Tomato	4.6	Turbidity at 36 hrs	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
		Gram smear of strept.	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
Peach	4.2	Turbidity at 36 hrs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Apricots	3.8	Turbidity at 36 hrs	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-
		Gram smear of strept.	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Sauerkraut	3.8	Turbidity at 36 hrs	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
		Gram smear of strept.	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Mayonnaise	3.7	Turbidity at 36 hrs	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 16.

			ATCC 1325 Days								ATCC 6057 Days							
			0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
Orange juice	3.5	Turbidity at 36 hrs	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-
		Gram smear of strept.	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-
Cranberry sauce	2.8	Turbidity at 36 hrs	/	-	-	-	-	-	-	-	/	-	-	/	/	-	-	-
		Gram smear of strept.	-	-	-	-	-	-	-	-	/	-	-	/	/	-	-	-
Apple sauce	3.6	Turbidity at 36 hrs	/	-	/	-	-	-	-	-	/	/	-	/	/	-	-	-
		Gram smear of strept.	/	-	-	-	-	-	-	-	/	/	/	/	/	/	/	/

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